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Endothelial Activation Biomarkers Increase after HIV-1 Acquisition: Plasma VCAM-1 Predicts Disease Progression

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Abstract

Objective—We aimed to determine whether endothelial activation biomarkers increase after HIV-1 acquisition, and whether biomarker levels measured in chronic infection would predict disease progression and death in HIV-1 seroconverters.

Design—HIV-1-seronegative Kenyan women were monitored monthly for seroconversion, and followed prospectively after HIV-1 acquisition.

Methods—Plasma levels of angiopoietins-1 and -2 (ANG-1, ANG-2) and soluble vascular cell adhesion marker-1 (VCAM-1), intercellular adhesion marker-1 (ICAM-1), and E-selectin were tested in stored samples from before infection, acute infection, and at two points during chronic infection. We used non-parametric tests to compare biomarkers before and after HIV-1 acquisition, and Cox proportional-hazards regression to analyze associations with disease progression (CD4 <200 cells/μL, Stage IV disease, or ART initiation) or death.

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Conflicts of Interest

WCL is listed as a co-inventor on a patent applied for by the University Health Network (Toronto, ON, Canada) to develop point-of-care tests for endothelial activation biomarkers in infectious diseases. All other authors report no conflict of interest.

Results—Soluble ICAM-1 and VCAM-1 were elevated relative to baseline in all post-infection periods assessed ($p<0.0001$). Soluble E-selectin and the ANG-2:ANG-1 ratio increased in acute infection ($p=0.0001$), and ANG-1 decreased in chronic infection ($p=0.0004$). Among 228 subjects followed over 1,028 person-years, 115 experienced disease progression or death. Plasma VCAM-1 levels measured during chronic infection were independently associated with time to HIV progression or death (aHR 5.36, 95% confidence interval 1.99–14.44 per $\log_{10}$ increase), after adjustment for set point plasma viral load, age at infection, and soluble ICAM-1 levels.

Conclusions—HIV-1 acquisition was associated with endothelial activation, with sustained elevations of soluble ICAM-1 and VCAM-1 post-infection. Soluble VCAM-1 may be an informative biomarker for predicting the risk of HIV-1 disease progression, morbidity, and mortality.

Keywords

HIV-1; VCAM-1; ICAM-1; angiopoietin-1; angiopoietin-2; E-selectin; endothelial activation

Introduction

Chronic HIV-1 infection has been associated with immune activation and inflammatory cytokine production [1]. Elevated biomarkers of inflammation and coagulopathy, including interleukin-6 (IL-6) and D-dimer, have been associated with death or disease progression in several prospective studies of adults receiving antiretroviral therapy (ART) [2–4]. Such biomarkers have been associated with vascular dysfunction [5], which frequently occurs in the setting of endothelial activation [6,7]. Increased biomarkers of endothelial activation, notably soluble intercellular adhesion molecule-1 (ICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1), have been reported in persons with chronic HIV infection relative to HIV-uninfected controls [8,9]. We and others have reported that levels of endothelial activation biomarkers including soluble ICAM-1, VCAM-1, and E-selectin and plasma angiopoietin-2 (ANG-2) decrease, but do not normalize, after ART initiation, and that levels of these biomarkers may also predict adverse outcomes [10–13].

While it is becoming clear that endothelial activation may be important in chronic HIV infection, much less is known about the effect of HIV-1 acquisition on this process. In addition, it is unknown whether biomarker levels measured early in HIV-1 infection, before immunosuppression occurs, may be predictive of adverse outcomes. We hypothesized that biomarkers of endothelial activation would increase after HIV-1 acquisition, and that plasma levels of these biomarkers during chronic infection would be associated with disease progression and death.

Methods

Ethics statement

All participants gave written informed consent. Ethical review committees of the Kenyatta National Hospital, University of Washington, and Fred Hutchinson Cancer Research Center approved the research protocols.

Study population and procedures

We included all women with a known date of HIV-1 seroconversion who were identified in the Mombasa high-risk women's cohort between 1993 and 2009, and for whom stored plasma samples were available to test endothelial activation biomarkers. Procedures for follow-up in this cohort have been described previously [14,15]. Briefly, HIV-1-seronegative women who attended a municipal clinic in Mombasa, Kenya and reported

engaging in transactional sex were invited to participate. At enrollment and monthly thereafter, women were interviewed using standardized questionnaires about recent sexual behavior and health status, then underwent a standardized physical examination. Blood was collected for HIV-1 serologic testing at each monthly visit.

Women who seroconverted to HIV-1 during follow-up were asked to continue their monthly clinic visits, and blood samples were obtained quarterly. Women who missed two consecutive clinic appointments were traced at their workplaces by trained study staff. For women who died, information on the date of death was collected from colleagues and employers during these tracing visits. The cause of death could be verified for only a limited number of participants.

At each visit, study participants received individualized, confidential risk-reduction counseling, free condoms, and general outpatient medical care including, for women who acquired HIV-1, cotrimoxazole prophylaxis, isoniazid preventive therapy for latent tuberculosis infection, and treatment of opportunistic infections. Beginning in 2004, antiretroviral therapy (ART) was offered in the research clinic, and also became available through other clinics in the Mombasa area. ART status was recorded at each study visit, and the date of ART initiation was ascertained for all participating women.

Laboratory methods

HIV-1 serostatus was evaluated using the Detect HIV1/2 ELISA (BioChem Immunosystems) confirmed by a second ELISA (Recombigen, Cambridge Biotech or Vironostika HIV-1 Uni-Form II Ag/Ab, bioMérieux). CD4 cell counts were determined using FACS Count (Becton Dickinson). Plasma specimens were frozen at -70°C until shipment to Seattle on dry ice or in liquid nitrogen for HIV-1 RNA quantitation using the Gen-Probe HIV-1 viral load assay [16]. The lower limit of quantitation was 100 copies/mL.

Date of HIV-1 infection

For women with HIV-1 detected in plasma samples collected before HIV-1 seroconversion, we estimated that HIV-1 infection occurred 17 days before the first sample was found to be positive for HIV-1 RNA, as in previous studies in this cohort [15]. For women who had no plasma viremia detected before HIV-1 seroconversion or for whom no pre-seroconversion samples were available for RNA testing, we estimated that HIV-1 infection occurred at the midpoint between the last clinic visit at which the woman was HIV-1 seronegative and the first clinic visit at which the woman was HIV-1 seropositive.

Endothelial activation biomarkers

Time periods for biomarker testing were defined as follows: the pre-infection period was the year prior to the estimated date of infection, the acute infection period was up to 119 days after the estimated date of infection, and chronic infection was ≥120 days after the estimated date of infection. Because we wanted to determine whether the endothelial biomarkers we studied were independently associated with outcomes after adjustment for the set point plasma viral load, we further divided chronic infection into two periods: the first period corresponded to the viral load set point period, which was defined as from 120 to 720 days (i.e., 4 to 24 months) after the estimated date of infection [17,18], and the second period was defined as >720 days after the estimated date of infection, when viral load may start to increase. Aliquots of the first available stored plasma samples (or last available, in the case of pre-infection) from these four time periods were shipped to Toronto on dry ice and stored at −80°C prior to testing for endothelial activation biomarkers [19,20].

Plasma concentrations (dilution factors indicated in parentheses) of angiopoietin-1 (ANG-1, 1:5), ANG-2 (1:5), soluble ICAM-1 (1:1000), soluble VCAM-1 (1:2000), and soluble E-selectin (1:50) were measured by ELISA (R&D Systems Duoset kits, Minneapolis MN, USA) according to manufacturers' instructions with modifications: (1) assays were performed in 50 μL per well; (2) plasma samples were incubated overnight at 4°C; and (3) ELISAs were developed using Extravidin®-Alkaline Phosphatase (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada; 1:1000 dilution, 45-minute incubation) followed by addition of p-nitrophenyl phosphate substrate (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada) before optical density reading at 405 nm. Concentrations were interpolated from 4-parameter-fit standard curves. Background levels were determined from blank wells included on each plate (assay buffer added instead of sample), and the subsequent optical density was subtracted from all samples and standards prior to analysis. Samples with optical densities below the lowest detectable standard were assigned the value of that standard. Lower limits of detection for each assay were as follows: ANG-1 – 19.53 pg/mL, 58.41 – ANG-2 pg/mL, s-ICAM-1 – 3.91 pg/mL, VCAM-1 – 1.95 pg/mL, and E-selectin – 11.72 pg/mL.

Statistical methods

Descriptive statistics were used to present data on characteristics at HIV-1 acquisition and timepoints for sample testing. The Skillings-Mack test (i.e., Friedman test when there are missing data) was used to conduct a global test of the null hypothesis that biomarker levels did not vary across periods [21,22]. Wilcoxon matched-pairs signed rank tests were used to compare endothelial activation biomarkers before infection to measurements in each period after HIV-1 acquisition. Spearman's rho was used to calculate non-parametric correlations between endothelial activation biomarkers and plasma HIV-1 RNA during the first chronic infection period. Wilcoxon rank sum tests were used to compare endothelial activation biomarkers measured during the first chronic infection period in women who did versus did not die or experience disease progression during follow-up.

Cox proportional hazards regression was used to analyze associations between endothelial activation biomarkers measured in the first chronic infection period (i.e., corresponding to the time of viral load set point) and time to disease progression (i.e., stage IV disease or CD4 count <200 cells/μL), ART initiation (recommended for stage IV disease or CD4 count <200 cells/μL during the study period), or all-cause mortality. For this analysis, follow-up time was measured from the collection date of the sample tested (to estimate the predictive value of biomarkers from this timepoint), and participants were censored at the last visit before loss to follow-up or the end of the study period, whichever occurred first. Multivariable analysis was used to adjust for potential confounders, including set point plasma viral load and age at infection.

A separate Cox proportional hazards regression was used to analyze associations between endothelial activation biomarkers measured later in chronic infection (i.e., after the viral load set point period) and time to disease progression, ART initiation, or all-cause mortality. For this analysis, follow-up time was measured from the collection date of the sample tested, and participants were censored at the last visit before loss to follow-up or the end of the study period, whichever occurred first. Multivariable analysis was used to adjust for potential confounders, including plasma viral load measured at the same timepoint in chronic infection and age at infection.

Data were analyzed using Stata version 11.2 (StataCorp, College Station, Texas). A two-sided p value <0.05 was considered significant.

Results

Study population

Characteristics of the 306 HIV-1 seroconverters who met the inclusion criteria are presented in Table 1. Of these women, 106 (34.6%) had plasma HIV-1 RNA detected prior to seroconversion. Plasma HIV-1 RNA results were available for 232 women (75.8%) during acute infection, 242 women (79.1%) in the first chronic infection period, and 203 women (66.3%) later in chronic infection. Of note, only 1 of 232 women (0.4%) had started ART during the first chronic infection period and only 10 of 159 women (6.3%) had started ART later in chronic infection. Excluding visits on which women were taking ART, median plasma HIV-1 RNA levels were 87,410 copies/mL (inter-quartile range [IQR], 7,321–696,012 copies/mL) during acute infection, 49,698 copies/mL (IQR, 8,740–160,040 copies/mL) in the first chronic infection period, and 46,839 copies/mL (IQR, 11,830–191,900 copies/mL) later in chronic infection.

Biomarker measurements

For the 162 women (52.9%) with pre-infection samples available, biomarkers were measured a median of 166 days (IQR, 89–293 days) prior to the estimated date of infection. For the 196 women (64.1%) with samples available during acute infection, biomarkers were measured a median of 48 days (IQR, 21–70 days) after the estimated date of infection. For the 232 women (75.8%) with samples available during the first chronic infection period, biomarkers were measured a median of 295 days (IQR, 187–471 days) after the estimated date of infection. Finally, for the 159 women (52.0%) with samples available later in chronic HIV-1 infection, biomarkers were measured a median of 1,358 days (IQR, 1,160–1,593 days) after the estimated date of infection. Of note, there were no differences between women with and without pre-infection biomarker measurements, with respect to the characteristics presented in Table 1.

Changes in endothelial activation biomarkers with HIV-1 acquisition and disease progression

Global tests of plasma ICAM-1, VCAM-1, and E-selectin levels over all four study periods revealed significant differences ($p<0.0001$ for each biomarker). Global tests of angiopoietin levels showed significant differences in plasma ANG-1 levels and the ratio of plasma ANG-2:ANG-1 across the four periods ($p=0.02$ for each comparison), but no significant change in plasma ANG-2. Table 2 presents median levels of each biomarker before infection, during acute infection, and in each chronic infection period. Increases in soluble ICAM-1 and soluble VCAM-1 were highly significant at all points after infection, while an increase in soluble E-selectin was limited to acute infection. Median plasma ANG-1 levels decreased steadily over the course of HIV-1 infection, with the difference from pre-infection baseline becoming significant during the second chronic infection period ($p=0.0004$). Although plasma ANG-2 levels increased over the course of HIV-1 infection and disease progression, there were no significant differences from the pre-infection baseline ($p>0.15$ in all periods). The plasma ANG-2:ANG-1 ratio, however, increased during acute infection ($p=0.003$, see table for details). These differences are depicted graphically in Figure 1.

Endothelial activation biomarkers during the viral load set point period

During the first chronic infection period, which corresponded to the viral load set point period, plasma viral load was positively correlated with soluble ICAM-1 and soluble VCAM-1 ($\rho=0.283$, $p<0.0001$ and $\rho=0.233$, $p=0.0004$ respectively), but not with plasma ANG-1, plasma ANG-2, or soluble E-selectin. Levels of soluble ICAM-1 and VCAM-1 were higher among women who experienced disease progression or death compared to

women who did not (median 240.4 ng/mL vs. 201.6 ng/mL, p=0.002; and median 619.3 ng/mL vs. 511.7 ng/mL, p=0.001, respectively). There were no significant differences in plasma ANG-1 levels, plasma ANG-2 levels, the ANG-2:ANG-1 ratio, or soluble E-selectin levels between women who died or progressed and those who did not.

VCAM-1 measured during the viral load set point period predicts time to disease progression, ART initiation, or death

Among 228 subjects with both biomarker and viral load measurements available during the set point period (i.e., the first chronic infection period) and subsequent event-free follow-up time, 115 experienced disease progression (i.e., CD4 count <200 cells/μL, Stage IV disease, ART initiation) or death. These 228 subjects contributed 1,028 person-years (py), during which the event rate was 11.2 per 100 py (95% CI, 9.3–13.4 per 100 py). Women who had both set point biomarker measurements and subsequent follow-up had a lower level of education and were less likely to use tobacco, but did not differ with respect to the other characteristics presented in Table 1. Ten women started ART prior to experiencing disease progression, of whom seven had a CD4 count nadir <300 cells/μL prior to ART initiation and three started ART at outside clinics during a break in follow-up and returned with higher CD4 counts.

Figure 2 presents the results of both bivariable and multivariable analyses of factors associated with the combined outcome. As expected, set point plasma viral load and age at infection were significant predictors of disease progression, ART initiation, or death. Both soluble ICAM-1 and soluble VCAM-1 were associated with the combined outcome in bivariable analysis. After adjustment for set point plasma viral load and age at infection, soluble VCAM-1 remained an independent predictor of time to HIV-1 disease progression or death (aHR 5.36, 95% confidence interval 1.99–14.44 per $\log_{10}$ increase, p=0.001). Soluble ICAM-1 was no longer a significant predictor in the multivariable model. In a sensitivity analysis excluding the 10 women who started ART prior to experiencing disease progression, results were unchanged.

Figure 3 illustrates the proportion of women who experienced disease progression or death according to soluble VCAM-1 categorized as greater than or equal to or below the median. Participants with both soluble VCAM-1 levels and plasma viral load greater than or equal to the median were at highest risk for an adverse outcome during follow-up.

Soluble VCAM-1 measured later in chronic infection also predicts time to disease progression, ART initiation, or death

One hundred and forty-five women had biomarkers measured later in chronic infection. Over 574 py from the time of these measurements, 35 women experienced disease progression or death, for a rate of 6.1 per 100 py (95% CI, 4.4–8.5 per 100 py). In this analysis, plasma viral load (measured at the same timepoint), soluble ICAM-1, soluble VCAM-1, and age at infection were associated with disease progression or death at p<0.10 in bivariable analysis. In a multivariable model including all these predictors, VCAM-1 was the only significant predictor, with an adjusted hazard of 4.67 (95% CI, 1.04–21.05) for each $\log_{10}$ increase in this biomarker.

Discussion

In this study of initially seronegative Kenyan women who acquired HIV-1 during follow-up, we have found that HIV-1 acquisition leads to endothelial activation, with increases in plasma levels of cellular adhesion biomarkers (soluble ICAM-1, VCAM-1, and E-selectin) and alterations in angiopoietin regulation in acute and chronic infection. Both soluble

ICAM-1 and soluble VCAM-1 remain elevated relative to pre-infection levels during all post-infection periods. We found that levels of soluble VCAM-1 measured during the set point period or later in chronic infection predicted time to disease progression or death, independent of plasma viral load.

VCAM-1 is a transmembrane immunoglobulin superfamily protein expressed by activated endothelial cells and smooth muscle cells [23]. VCAM-1 binds to very late antigen 4 (VLA-4), an integrin dimer expressed by monocytes, lymphocytes, and eosinophils, and promotes cell-cell adhesion and transmigration of inflammatory cells [23–25]. Both VCAM-1 and ICAM-1 are upregulated by inflammatory cytokines such as interleukin-1β and tumor necrosis factor alpha, as well as C-reactive protein (CRP) produced by the liver in response to interleukin-6 [24]. VCAM-1 expression is restricted to endothelial and dendritic cells, whereas ICAM-1 is more broadly expressed [25]. The soluble forms of these adhesion molecules can be shed from cell surfaces and reflect the level of cellular activation [26].

Elevated levels of soluble ICAM-1 and soluble VCAM-1 have been reported in HIV-infected individuals compared to healthy controls in a number of studies [8,9,27–30]. These elevations have been associated with higher levels of viral replication [9], and were independent of CRP levels and inconsistently related to CD4 cell counts [8,9,27]. VCAM-1 levels remained elevated in untreated persons with chronic infection, but were reduced after up to 24 months of ART [28], suggesting that chronic HIV-1 infection, not its pharmacological treatment, induces endothelial activation. Although soluble adhesion molecules, especially soluble ICAM-1, may be expressed by other cell types, including fibroblasts and leukocytes [26], these findings are consistent with in vitro studies showing that viral *Tat* protein induces VCAM-1 expression in human pulmonary artery endothelial cells [31].

Elevations in VCAM-1 levels have been reported in adolescents infected with HIV-1 through adult risk behavior, suggesting that endothelial activation occurs in response to HIV-1 acquisition even in young individuals [32]. To our knowledge, our study is the first to measure endothelial activation biomarkers before and after HIV-1 acquisition and to prospectively evaluate the association between endothelial activation biomarkers and disease progression in HIV-1 seroconverters. Of note, soluble VCAM-1 has been found to predict risk of future cardiovascular death in HIV-1-uninfected populations, independently of classic risk factors and CRP levels [33]. We have no data on cardiovascular outcomes in our study population, but additional research to investigate the relationship between endothelial activation biomarkers and future cardiovascular disease outcomes in HIV-1-infected patients is warranted, based on our findings.

In our completed study of changes in endothelial activation biomarkers among chronically infected Kenyan women initiating ART, plasma levels of soluble ICAM-1, soluble VCAM-1, soluble E-selectin, and plasma ANG-2 decreased after treatment, with a concomitant increase in plasma ANG-1 levels [10]. While decreased plasma levels of soluble ICAM-1 and a decreased ANG-2:ANG-1 ratio were seen at months 6 and 12, a decrease in soluble VCAM-1 was not seen until 12 months after ART initiation. Baseline plasma ANG-2 and soluble ICAM-1, but not soluble VCAM-1, predicted mortality after ART initiation [10]. It is possible that increased plasma ANG-2 and soluble ICAM-1 levels in that study of women with advanced HIV-1 infection were associated with undiagnosed co-infections, such as tuberculosis. In the current study of HIV-1 seroconverters, increased soluble VCAM-1 levels measured early in chronic infection, at the time of viral load set point, were unlikely to be due to opportunistic disease.

An important strength of this study is that participants were followed longitudinally before and after HIV-1 acquisition. In addition, the time period of this study included many years prior to the availability of antiretroviral therapy in Kenya, providing insight into the natural history of HIV-1-related endothelial activation. We were able to study disease progression among 228 HIV-1 seroconverters who had samples available in the set point period and subsequent monitoring for disease progression. The long follow-up time and large number of events provided adequate power to detect important differences in outcomes.

Our study has a number of limitations. First, we had incomplete data due to missing samples and loss to follow-up. In particular, only 162 women (35%) had stored pre-infection samples available for testing. Nevertheless, we found no differences between women with and without pre-infection biomarker measurements. Second, we had incomplete data on potential confounding factors, including intervening infections, nutritional status, substance use, and chronic conditions such as diabetes and hypertension, which may be related to endothelial activation. Third, there are no clinically relevant cut-points for endothelial activation biomarkers, and assays are not standardized, which makes it difficult to compare studies directly. Fourth, we did not measure other known biomarkers such as high sensitivity CRP, fibrinogen, D-dimer, and interleukin 6, and so could not determine their association with biomarkers of endothelial activation in this population. However, these four biomarkers were not found to be elevated after HIV-1 acquisition in a cohort of male seroconverters [34]. Finally, because we studied a group of Kenyan women who had seroconverted for HIV-1 infection, our results may not be generalizable to men, to persons in other geographic locations, or to persons who are treated with ART early in the course of infection.

In conclusion, we found that HIV-1 acquisition led to endothelial activation, with sustained elevations of soluble ICAM-1 and soluble VCAM-1. There was evidence of angiopoietin dysregulation in acute infection, with an increased ANG-2:ANG-1 ratio, and in chronic infection, with decreases in plasma ANG-1. Soluble VCAM-1 measured during the viral load set point period was a strong predictor of adverse outcomes, independent of set point plasma viral load, and was also associated with outcomes when measured later in chronic infection. HIV-1 infection increases plasma levels of soluble VCAM-1, which may be an informative biomarker for predicting HIV-1 disease progression, morbidity, and mortality.

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SMG, WCL, and JO conceived and designed the experiments; RSM, WJ, BBAE, and SMG performed or supervised field work; NR and WCL performed or supervised laboratory testing; and SMG analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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References

1. Nixon DE, Landay AL. Biomarkers of immune dysfunction in HIV. Curr Opin HIV AIDS. 2010; 5:498–503. [PubMed: 20978393]
2. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008; 5:e203. [PubMed: 18942885]
3. Boulware DR, Hullsiek KH, Puronen CE, Rupert A, Baker JV, French MA, et al. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. J Infect Dis. 2011; 203:1637–1646. [PubMed: 21592994]
4. Ledwaba L, Tavel JA, Khabo P, Maja P, Qin J, Sangweni P, et al. Pre-ART levels of inflammation and coagulation markers are strong predictors of death in a South African cohort with advanced HIV disease. PLoS One. 2012; 7:e24243. [PubMed: 22448211]
5. Baker J, Quick H, Hullsiek KH, Tracy R, Duprez D, Henry K, et al. Interleukin-6 and d-dimer levels are associated with vascular dysfunction in patients with untreated HIV infection. HIV Med. 2010; 11:608–609. [PubMed: 20456504]
6. Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, Piera K, et al. Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria. Proc Natl Acad Sci U S A. 2008; 105:17097–17102. [PubMed: 18957536]
7. Davis JS, Yeo TW, Piera KA, Woodberry T, Celermajer DS, Stephens DP, et al. Angiopoietin-2 is increased in sepsis and inversely associated with nitric oxide-dependent microvascular reactivity. Crit Care. 2010; 14:R89. [PubMed: 20482750]
8. Gattegno L, Bentata-Peyssare M, Gronowski S, Chaouche K, Ferriere F. Elevated concentrations of circulating intercellular adhesion molecule 1 (ICAM-1) and of vascular cell adhesion molecule 1 (VCAM-1) in HIV-1 infection. Cell Adhes Commun. 1995; 3:179–185. [PubMed: 8846020]
9. Calza L, Pocaterra D, Pavoni M, Colangeli V, Manfredi R, Verucchi G, et al. Plasma levels of VCAM-1, ICAM-1, E-Selectin, and P-Selectin in 99 HIV-positive patients versus 51 HIV-negative healthy controls. J Acquir Immune Defic Syndr. 2009; 50:430–432. [PubMed: 19322038]
10. Graham, S.; Rajwans, N.; Tapia, K.; Jaoko, W.; Estambale, B.; McClelland, S., et al. Angiopoietin-2 is elevated in advanced HIV-1 infection, decreases after antiretroviral therapy initiation, and is related to plasma HIV-1 RNA. 19th Conference on Retroviruses and Opportunistic Infections; Seattle, Washington. 2012; Abstract 281
11. Wolf K, Tsakiris DA, Weber R, Erb P, Battegay M. Swiss HIV Cohort Study. Antiretroviral therapy reduces markers of endothelial and coagulation activation in patients infected with human immunodeficiency virus type 1. J Infect Dis. 2002; 185:456–462. [PubMed: 11865397]
12. Papasavvas E, Azzoni L, Pistilli M, Hancock A, Reynolds G, Gallo C, et al. Increased soluble vascular cell adhesion molecule-1 plasma levels and soluble intercellular adhesion molecule-1 during antiretroviral therapy interruption and retention of elevated soluble vascular cellular adhesion molecule-1 levels following resumption of antiretroviral therapy. AIDS. 2008; 22:1153–1161. [PubMed: 18525261]
13. Sipsas NV, Sfikakis PP, Touloumi G, Pantazis N, Choremi H, Kordossis T. Elevated serum levels of soluble immune activation markers are associated with increased risk for death in HAART-naive HIV-1-infected patients. AIDS Patient Care STDS. 2003; 17:147–153. [PubMed: 12737638]
14. Martin HL Jr, Jackson DJ, Mandaliya K, Bwayo J, Rakwar JP, Nyange P, et al. Preparation for AIDS vaccine evaluation in Mombasa, Kenya: establishment of seronegative cohorts of commercial sex workers and trucking company employees. AIDS Res Hum Retroviruses. 1994; 10 (Suppl 2):S235–237. [PubMed: 7865309]
15. Lavreys L, Baeten JM, Kreiss JK, Richardson BA, Chohan BH, Hassan W, et al. Injectable contraceptive use and genital ulcer disease during the early phase of HIV-1 infection increase plasma virus load in women. J Infect Dis. 2004; 189:303–311. [PubMed: 14722896]
16. Emery S, Bodrug S, Richardson BA, Giachetti C, Bott MA, Panteleeff D, et al. Evaluation of performance of the Gen-Probe human immunodeficiency virus type 1 viral load assay using primary subtype A, C, and D isolates from Kenya. J Clin Microbiol. 2000; 38:2688–2695. [PubMed: 10878065]

17. Sagar M, Lavreys L, Baeten JM, Richardson BA, Mandaliya K, Chohan BH, et al. Infection with multiple human immunodeficiency virus type 1 variants is associated with faster disease progression. J Virol. 2003; 77:12921–12926. [PubMed: 14610215]
18. Lavreys L, Baeten JM, Chohan V, McClelland RS, Hassan WM, Richardson BA, et al. Higher set point plasma viral load and more-severe acute HIV type 1 (HIV-1) illness predict mortality among high-risk HIV-1-infected African women. Clin Infect Dis. 2006; 42:1333–1339. [PubMed: 16586394]
19. Ricciuto DR, dos Santos CC, Hawkes M, Toltl LJ, Conroy AL, Rajwans N, et al. Angiopoietin-1 and angiopoietin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis. Crit Care Med. 2011; 39:702–710. [PubMed: 21242795]
20. Page AV, Kotb M, McGeer A, Low DE, Kain KC, Liles WC. Systemic dysregulation of angiopoietin-1/2 in streptococcal toxic shock syndrome. Clin Infect Dis. 2011; 52:e157–161. [PubMed: 21460306]
21. Durbin J. Incomplete blocks in ranking experiements. Br J Psychol. 1951; 4:85–90.
22. Skillings JH, Mack GA. On the use of a Friedman-type statistic in balanced and unbalanced block designs. Technometrics. 1981; 23:171–177.
23. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA–4 at a site distinct from the VLA-4/ fibronectin binding site. Cell. 1990; 60:577–584. [PubMed: 1689216]
24. Szmitko PE, Wang CH, Weisel RD, de Almeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation: Part I. Circulation. 2003; 108:1917–1923. [PubMed: 14568885]
25. Gearing AJ, Hemingway I, Pigott R, Hughes J, Rees AJ, Cashman SJ. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance. Ann N Y Acad Sci. 1992; 667:324–331. [PubMed: 1285023]
26. Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis. 2003; 170:191–203. [PubMed: 14612198]
27. Nordoy I, Aukrust P, Muller F, Froland SS. Abnormal levels of circulating adhesion molecules in HIV-1 infection with characteristic alterations in opportunistic infections. Clin Immunol Immunopathol. 1996; 81:16–21. [PubMed: 8808636]
28. Francisci D, Giannini S, Baldelli F, Leone M, Belfiori B, Guglielmini G, et al. HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. AIDS. 2009; 23:589–596. [PubMed: 19177019]
29. Fourie C, van Rooyen J, Pieters M, Conradie K, Hoekstra T, Schutte A, et al. Is HIV-1 infection associated with endothelial dysfunction in a population of African ancestry in South Africa? Cardiovasc J Afr. 2011; 22:134–140. [PubMed: 21713302]
30. Melendez MM, McNurlan MA, Mynarcik DC, Khan S, Gelato MC. Endothelial adhesion molecules are associated with inflammation in subjects with HIV disease. Clin Infect Dis. 2008; 46:775–780. [PubMed: 18225982]
31. Liu K, Chi DS, Li C, Hall HK, Milhorn DM, Krishnaswamy G, et al. HIV-1 Tat protein-induced VCAM-1 expression in human pulmonary artery endothelial cells and its signaling. Am J Physiol Lung Cell Mol Physiol. 2005; 289:L252–260. [PubMed: 15805138]
32. Syed SS, Balluz RS, Kabagambe EK, Meyer WA 3rd, Lukas S, Wilson CM, et al. Assessment of biomarkers of cardiovascular risk among HIV-1-infected adolescents: role of soluble Vascular Cell Adhesion Molecule (sVCAM) as an early indicator of endothelial inflammation. AIDS Res Hum Retroviruses. 2012 (Epub ahead of print).
33. Blankenberg S, Rupprecht HJ, Bickel C, Peetz D, Hafner G, Tiret L, et al. Circulating cell adhesion molecules and death in patients with coronary artery disease. Circulation. 2001; 104:1336–1342. [PubMed: 11560847]
34. Achhra AC, Amin J, Law MG, Grulich AE, Yeung J, Kelleher AD, et al. Changes in metabolic, inflammatory and coagulation biomarkers after HIV seroconversion –the Health in Men (HIM) Biomarker sub-study. Antivir Ther. 2012 (Epub ahead of print).

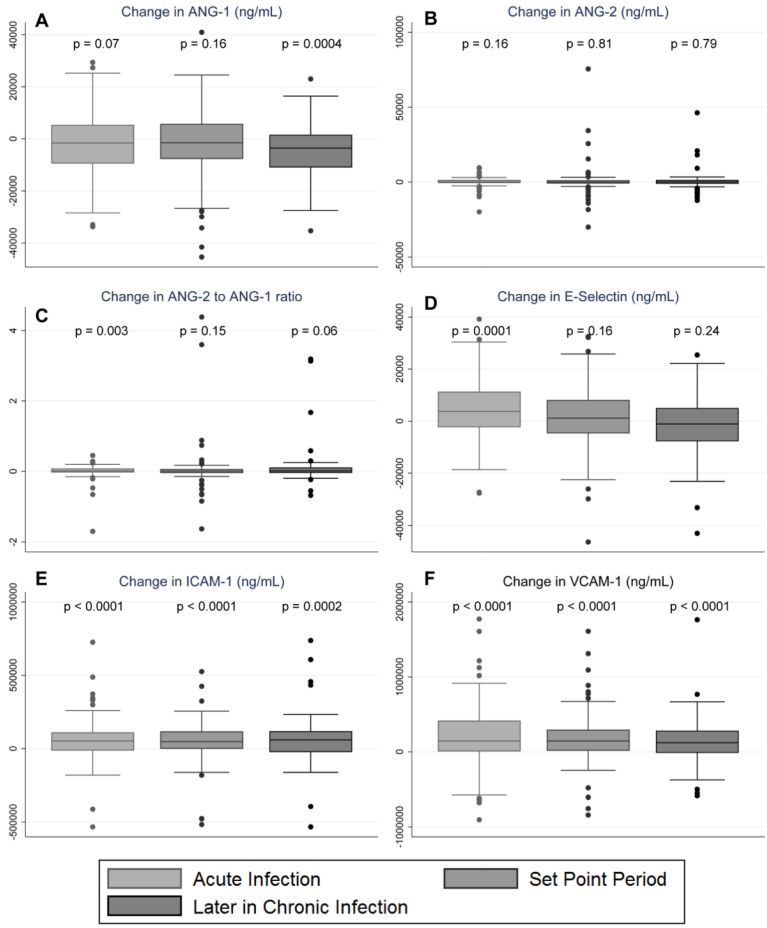

Figure 1. Change in Endothelial Activation Biomarkers among Kenyan Women Who Acquired HIV-1 Infection

Box plots depict the 75th percentile (upper hinge), median (solid line), and 25th percentile (lower hinge) of the difference from baseline for each biomarker at each timepoint. The lower and upper whiskers are the lowest datum still within 1.5 inter-quartile range of the lower quartile, and the highest datum still within 1.5 inter-quartile range of the upper quartile, respectively. Outlier values (outside the whiskers) are indicated. Timepoints are color coded according to the Legend, and presented in sequence (i.e., acute infection, set point period, later in chronic infection). P values are from paired non-parametric comparisons (i.e., Wilcoxon matched-pairs signed rank tests) with baseline, as in Table 2. ANG-1 = plasma angiopoietin-1, ANG-2 = plasma angiopoietin-2, E-selectin = soluble E-selectin, ICAM-1 = soluble intercellular adhesion molecule-1, VCAM-1 = soluble vascular cell adhesion molecule-1.

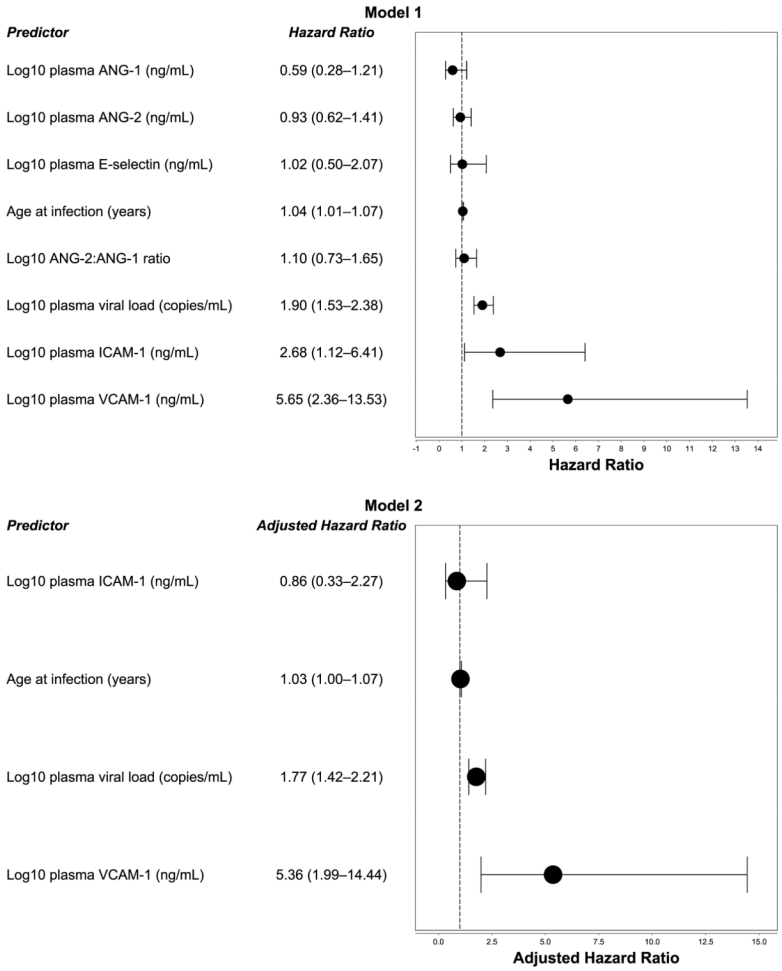

Figure 2. Cox Regression of Time from Biomarker Measurements in the Viral Load Set Point Period to HIV-1 Disease Progression or Death

Cox regression was used to evaluate the hazard of disease progression (i.e., CD4 count <200 cells/μL, Stage IV disease, ART initiation) or death among 228 subjects with both set point period biomarker and viral load measurements available. Forest plots are used to present the hazard ratios from bivariable analysis (Model 1) and the adjusted hazard ratios from multivariable analysis (Model 2). The black circle indicates the point estimate and the whiskers indicate the 95% confidence intervals for each estimate. ANG-1 = angiopoietin-1, ANG-2 = angiopoietin-2, E-selectin = soluble E-selectin, ICAM-1 = soluble intercellular adhesion molecule-1, VCAM-1 = soluble vascular cell adhesion molecule-1.

N.B.: Potential confounders including hormonal status at infection, hormonal status at each visit, alcohol use, and tobacco use were not associated with the outcome at $p<0.10$ in bivariable analysis, and therefore are not included in the table.

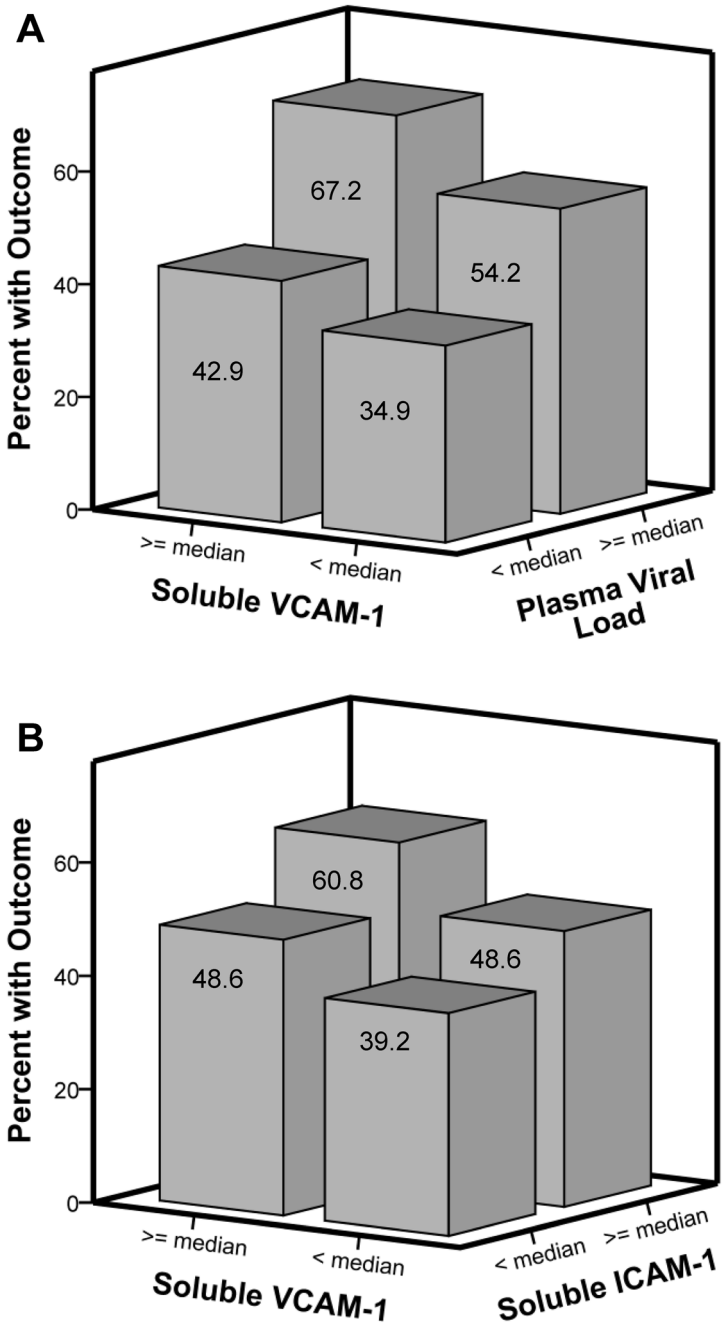

Figure 3. Percent of Women with Adverse Outcomes after Viral Load Set Point

Proportion of women who experienced disease progression or death (primary outcome) after the biomarker measurements during the viral load set point period, according to soluble VCAM-1 level greater than or equal to or below the median level (560,958 ng/mL), by plasma viral load (panel A) or soluble ICAM-1 (panel B) greater than or equal to or below the median level (49,698 copies/mL or 220,848 ng/mL, respectively). The group of women with both plasma viral load and soluble VCAM-1 greater than or equal to the median had the highest proportion of adverse outcomes (67.2%). ANG-1 = plasma angiopoietin-1, ANG-2 = plasma angiopoietin-2, E-selectin = soluble E-selectin, ICAM-1 = soluble intercellular adhesion molecule-1, VCAM-1 = soluble vascular cell adhesion molecule-1.

Table 1

Chacteristics of 306 Kenyan Women Who Acquired HIV-1 Infection.

Characteristic	Subcategory	All Women (N = 306)	Women with a pre-infection sample (N = 162)	Women without a pre- infection sample (N = 144)	Women with a set point sample and follow-up (N = 228)	Women without a set point sample and follow-up (N = 78)
		N (%) or Median (IQR)	*N (%) or Median (IQR)*	*N (%) or Median (IQR)*	*N (%) or Median (IQR)*	*N (%) or Median (IQR)*
Years of education*		8 (7–10)	8 (7–10)	7 (6–9)	8 (7–9)	8 (7–11)†
Marital status*	Never married	145 (47.4)	80 (49.4)	65 (45.1)	102 (44.7)	43 (55.1)
	Currently married	4 (1.3)	2 (1.2)	2 (1.4)	3 (1.3)	1 (1.3)
	Widowed or divorced	157 (51.3)	80 (49.4)	77 (53.5)	123 (53.9)	34 (43.6)
Workplace*	Bar/restaurant/guesthouse	258 (84.3)	132 (81.5)	126 (87.5)	191 (83.8)	67 (85.9)
	Nightclub	35 (11.4)	21 (13.0)	14 (9.7)	26 (11.4)	9 (11.5)
	Home-based or other	13 (4.3)	9 (5.5)	4 (2.8)	11 (4.8)	2 (2.6)
Alcohol use*	None	61 (19.9)	31 (19.1)	30 (20.8)	40 (17.5)	21 (26.9)
	1–7 drinks per week	113 (37.0)	68 (42.0)	45 (31.2)	87 (38.2)	26 (33.3)
	8–14 drinks per week	55 (17.9)	27 (16.7)	28 (19.4)	43 (18.9)	12 (15.4)
	>14 drinks per week	77 (25.2)	36 (22.2)	41 (28.5)	58 (25.4)	19 (24.4)
Tobacco use*		37 (12.1)	17 (10.5)	20 (13.9)	22 (9.6)	15 (19.2)†
Sex partners, past week**		1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)
Hormonal status**	No hormonal contraceptive use	160 (52.3)	81 (50.0)	79 (54.9)	116 (50.9)	44 (56.4)
	Oral contraceptive pills	37 (12.1)	18 (11.1)	19 (13.2)	31 (13.6)	6 (7.7)
	Depot Medroxyprogesterone acetate	98 (32.0)	56 (34.6)	42 (29.2)	74 (32.5)	24 (30.8)
	Norplant	4 (1.3)	4 (2.5)	0	3 (1.3)	1 (1.3)
	Pregnant	7 (2.3)	3 (1.9)	4 (2.8)	4 (1.8)	3 (3.8)
Age at infection		29 (25–34)	29 (25–34)	29 (25–34)	29 (25–35)	27 (24–32)
Days from EDI to seroconversion		66 (45–175)	57 (42–115)	86 (47–289)	69 (45–174)	60 (44–187)

EDI = estimated day of infection, IQR = inter-quartile range

* Reported at cohort enrollment. Of note, no cocaine, amphetamine, or injection drug use was reported.

† p value < 0.05 for comparison.

** Reported at the seroconversion visit.

Table 2

Changes in Biomarkers of Endothelial Activation Before and After HIV-1 Acquisition.

Time Period	**Pre-Infection**	**Acute Infection**	**Chronic Infection during Set Point Period**	**Later in Chronic Infection**
N	**162**	**196**	**232**	**159**
Biomarker	***Median (IQR)***	***Median (IQR)***	***Median (IQR)***	***Median (IQR)***
Angiopoietin-1 (ng/mL)	21.7 (16.2–32.1)	19.7 (11.8–27.4) *P = 0.07*	18.4 (13.2–26.2) *P = 0.16*	17.1 (12.9–23.4) *P = 0.0004*
Angiopoietin-2 (ng/mL)	1.7 (0.9–3.4)	2.0 (1.3–3.4) *P = 0.16*	2.0 (1.1–3.7) *P = 0.81*	2.2 (1.3–3.4) *P = 0.79*
ANG-2: ANG-1 ratio	0.086 (0.039–0.145)	0.116 (0.059–0.256) *P = 0.003*	0.125 (0.060–0.216) *P = 0.15*	0.133 (0.069–0.230) *P = 0.06*
ICAM-1 (ng/mL)	170.1 (130.2–221.8)	238.0 (179.2–293.7) *P < 0.0001*	220.8 (174.0–286.1) *P < 0.0001*	234.7 (177.8–286.7) *P = 0.0002*
VCAM-1 (ng/mL)	402.0 (278.4–508.4)	563.7 (412.6–803.4) *P < 0.0001*	561.0 (400.0–791.1) *P < 0.0001*	576.8 (425.4–811.0) *P < 0.0001*
E-selectin (ng/mL)	17.6 (11.7–26.9)	25.8 (16.4–32.5) *P = 0.0001*	20.7 (13.2–29.0) *P = 0.16*	19.1 (11.6–25.5) *P = 0.24*

ANG-1 = angiopoietin-1, ANG-2 = angiopoietin-2, ICAM-1 = soluble intercellular adhesion molecule-1, VCAM-1 = soluble vascular cell adhesion molecule-1.

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